International Journal of Bio-Technology
and Research (IJBTR)
ISSN(P): 2249-6858; ISSN(E): 2249-796X
Vol. 5, Issue 2, Apr 2015, 17-26

ENDOPHYTES: A SOURCE OF BIOACTIVE METABOLITES

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ABSTRACT

There is a continuous need to search an alternative source for potential natural bioactive agents for curing of various types of diseases. Comparison between bioactive compounds obtained from the plants, microbes and endophytes were studied. But the most promising source and an extremely unusual organic molecules which proved to be valuable are produced by the endophytes. They are sources of novel chemistry and biology studies which will help to solve the problems in human and plant.

KEYWORDS: Bioactive Metabolites, Endophytes, Secondary Metabolites, Microbes

INTRODUCTION

Plants, microorganisms and animals have provided to mankind a large variety of biologically active compounds which have found diverse applications in health and curing diseases since the very beginning of human civilization. Initially, plants and other natural sources were the main parts of folk medicines practiced by the ancient man in different parts of the world. They gave rise to the traditional system of medicine. From folk medicine and traditional systems of medicine, medicinal and aromatic plants and other natural products were adopted into the modern system of medicine after they had been found as effective drugs through chemical and pharmacological screening. In the initial stages of development of modern medicine, plants and plant products formed an important part of pharmacopoeia. However, because of significant development in synthetic drug chemistry and antibiotics, there was a certain amount of decline in the use of plants in modern medicine and at one time, one would have thought that ultimately chemist will be able to synthesize all the active constituents of plants which are required by the modern medicine. Since modern medicine has not been able to provide cure to some of the diseases like cancer, AIDS, cardiovascular diseases, arthritis, etc the future of mankind is partially dependent for their health care needs on medicinal and aromatic plants growing on different parts of the world.

Natural product chemistry has witnessed over the years, discoveries of novel molecules by organic chemists, working on different products of metabolism from the biological kingdom. Plants and microorganisms have provided to mankind a large variety of biologically active compounds such as terpenoids, steroids, flavonoids, phenols, quinoles alkaloids and peptides which have found diverse applications in health care needs. In addition to food, clothing and shelter, plants have also provided all the medicaments for man and his domestic animals for thousands of years.

DIVERSITY OF BIOACTIVE METABOLITES

Plants have provided dyes, perfumes, spices, poisons, cosmetics and several well known medicines such as taxol, vinblastine, vincristine etc. Microbes are also known to produce a range of low volume and high value drugs such as hallucinogens, ergot alkaloids, vinblastine, vincristine and taxol. These compounds are often restricted to a narrow set of species within a phylogenetic group. Thus, they are species-specific and are not directly referable to the essential metabolic functions associated with growth of the concerned species. In other words, these compounds are "secondary" in origin and distinct from primary products of metabolism responsible for the synthesis of the various macromolecules responsible for the normal growth process. Bu'lock (1961) was the first to recognize the diversity of naturally occurring metabolites in the biological systems.[1] Since these molecules were different and more complex than those involved in primary metabolic pathways, he introduced the term secondary metabolites to characterize them. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms. They possess highly complex structures arising through the action of multiple enzyme system which are regulated by multiple genes. Unlike primary metabolites, absence of secondary metabolites results not in immediate death, but in long-term impairment of the organism's survivability or aesthetics, or perhaps in no significant change at all. Since the early days of mankind, plants with secondary metabolites have been used by humans to treat infections, health disorders and illness. Only during the last 100 years, have natural products been partly replaced by synthetic drugs, for which plant structures were a lead in many instances (e.g. salicylic acid for aspirin). The use of plant drugs for medical treatment is possible since plants have evolved bioactive secondary metabolites that have been selected during evolution as a means against various diseases. On a global scale, medicinal plants are mainly used as crude drugs and extracts. However, with the discovery of endophytic microbes several of the more potent and active substances are isolated from endophytic fungus.

The function or importance of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc). A number of therapeutically useful low volume and high valued life saving drugs such as taxol, and other bioactive molecules have been isolated from plants, microbes, and plant associated microbes and endophytic fungi.

BIOACTIVE METABOLITES FROM PLANTS

The healing powers of plants or their extracts have been used since ancient times for different ailments and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin) etc. In addition to the above drugs, plants have also provided dyes, perfumes, spices, poisons, cosmetics etc. The total number of natural products produced by plants has been estimated at over 500,000 [2]. Plant cells produce two types of metabolites. Primary metabolites are involved directly in growth and metabolism, viz. carbohydrates, lipids and proteins. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity viz. alkaloids, phenolics, essential oils, terpenes, sterols, flavonoids, lignins, tannins, etc. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides [3,4,5,6]. Seven plant-derived drugs currently used clinically for various types of cancers are taxol from *Taxus* species, vinblastine and vincristine from *Catharanthus roseus*, topotecan and irinotecan from *Camptotheca accuminata*, and etoposide and teniposide from *Podophyllum peltatum*[6]. Paclitaxel (Taxol), a tubulin binding diterpenoid was first isolated from the pacific Yew tree *Taxus brevifolia*[7]. Because of its ability to bind

specifically to B-tubulin and its cytotoxicity at lower concentrations, it is being used for the treatment of several classical tumors. It was approved by USDA for clinical use in ovarian cancer and breast cancer. *Comptotheca acuminate*, a medicinal plant specifically distributed in China is rich in the anticancer compound such as topotecan, irinotecan and camptothecin. The drug mayapple, often called Padophyllum is obtained from rhizomes and roots of *Podophyllum peltatum*. Active constitutents of the plants are present in rhizomes in the form of resin called podophyllin/podophyllotoxin[8]. The important compounds of *Podophyllum peltatum* are etoposide and teniposide. *Calophyllum inophyllum*, a woody plant is medicinally important because of the presence of several classes of bioactive compounds such as anti-human immuno-deficiency virus (HIV) dipyranocoumarins[9]. The inophyllums and (+) calanolide isolated from *Calophyllum inophyllum* showed strong activity against HIV-1. Artimisinin, an endoperoxide produced by aerial parts of *Artemisia annua* is an effective against sensitive and multidrug resistant strains of *Plasmodium*, the malarial agent with little or no toxicity to human[10]. *Phyllanthus amarus*, is a potential plant for the treatment of hepatitis B by suppressing the growth and replication of the virus[11]. Phyllanthin and hypophyllanthin present in *Phyllanthus amarus* are also reported as hepatoprotective agents and protect hepatocytes against carbon tetrachloride (CCl4) and galactosamine induced cytotoxicity in rats[12]. Leaves and seeds of *Garcinia dulcis* have been used for the treatment of lymphatitis, parotitis and struma[13]. Opium poppy (*Papaver somniferum*) was one of the most important plants used by the ancient man all over the world. Opium contains more than 2 dozen alkaloids. The main alkaloids present in the opium and used in medicine are morphine, codeine, papaverine and thebaine etc. Egyptian Henbane (*Hyoscyamus muticus*) was used by Egyptians more than 400 years ago. It has been adopted in modern medicine only recently. The drug is obtained from leaves and flowering tops of the plant *Hyoscyamus muticus*. It is mainly used as a raw material for the production of hyoscyamine, hyoscine and atropine. Leaves of corkwood tree *Duboisia myoporoides* contain a number of tropane alkaloids. The most important alkaloids are hyoscine and hyoscyamine. Tobacco alkaloids nicotine and nornicotines are also found. More than 500 alkaloids have been reported from *Rauvolfia serpentina*. The most important alkaloids from this plant which are used in medicine are reserpine, rescinnamine and deserpidine[8].

*Panax quinquefolium (*Ginseng) has been used in Chinese medicine for more than 400 years. Ginseng contains a number of chemical compounds. However, the activity is due to a number of saponins termed ginsenosides. The plant *Dioscorea* contains steroidal sapogenin, diosgenin in their roots. Diosgenin itself is not a medicinal product, but it is one of the most important raw materials used for the synthesis of steroidal drugs, which include corticosteroids, sex hormones, oral contraceptives and anabolic steroids. Senna (*Cassia angustifolia*) leaves and pods contain anthraquinone glycosides, the important ones are sennosides A and B which are used in medicine. The husk of the psyllium seed (*Plantago ovata*) contains colloidal mucilage mainly consisting of xylose, arabinose, galacturonic acid with rhannose and galactose.

Overexploitation of plants, particularly roots, tubers and bark when used for commercial purposes, particularly drugs has endangered 4,000 to 10,000 species of medicinal plants[14]. Pharmaceutical industries are interested in getting plant based drugs from a microbial source in order to get rid of geographical and political barriers as well as environmental conditions. Thus, from a practical point of view, microbial fermentation as a means of producing drugs or bioactive substances has several advantages.

BIOACTIVE METABOLITES FROM MICROBES

Microbial sources have proved to be goldmines of bioactive metabolites and with the discovery of therapeutically useful compounds starting with penicillin, the search for diverse novel molecules with biological activity is intensified. It is

well known, that actinomycetes (Gram positive bacteria) have proved to be the most versatile group of microorganisms as far as synthesis of secondary metabolites are concerned. In recent years there has been a major focus upon the screening of fungal sources for novel secondary metabolites. Pearce (1997) gave a comprehensive review on bioactive fungal metabolites[15].

Penicillin and cephalosporin are the two most important and widely used antibacterial antibiotics produced by fungi. Recent advances in penicillin therapy including the production of semisynthetic derivatives with wider antibacterial spectra have been reviewed extensively in literature. Penalva *et al*., have reviewed the optimization of penicillin biosynthesis in fungi[16].

Penicillin and other related compounds have been reported from other fungi and this topic has been reviewed in detail by Lechevalier[17]. Presently, a large number of useful antibacterial antibiotics have been discovered from prokaryotes, primarily the actinomycetes. Griseofulvin is a clinically important antibiotic produced by fungi and used in the treatment of fungal infections. The properties, biosynthesis, and fermentation of griseofulvin have been reviewed. It has been used as an effective antibiotic against fungal infections, especially dermatomycosis of animals and humans by oral therapy[18].

Anticancer and antitumor activity of diverse mould metabolites with varying degrees of effectiveness in combating the disease and different levels of toxicity to the normal healthy cells have been reported in literature. Novel cell cycle inhibitors termed tryptostatins have been isolated from *Aspergillus flavus*. While compounds inhibiting metastasis have been identified from the fungus *Nattrassia mangferae*. The enzyme farresyltransferase is involved in the process and the inhibition of this enzyme would be a potential target for anticancer activity. A number of fungal metabolites including gliotoxin, andrastins, kurasoins, and fusidinol have exhibited this activity. Kurasoins are products of *Paecilomyces*[19]. While fusidienol has been isolated from *Fusidium griseum*[20].

Fungi as sources for novel antifungal agents have been screened and among the many compounds isolated from fungi the echinocandins and the pneumocandins are highly potent and promising and are currently in clinical trials. The echinocandins are produced by *Aspergillus nibulans* and are lipopeptides which are inhibitory to B-1, 3 glucan synthesis. This compound has shown activity against both candida and pneumocytis carinii, which is the causal agent of pneumonia in immunocompromised (especially AIDS) patients. Schwartz *et al*., 1989 reported the isolation of a new antifungal lipopeptide similar to echinocandin B from a fungus, *Zalerion arboricola* [21].

One of the interesting developments has been the discovery of fungal compounds which are inhibitors of squalene synthesis and are also potent antifungal agents. Zaragozic acids and squalestatins independently discovered by Glaxo and Merck laboratories are both inhibitors of squalene synthetase and also find application in the lowering of serum cholesterol levels. Bills *et al*., 1994 have given an account of the distribution of zaragozic acids using fungi[22]. As a result of screening fungi for hypocholesterolemic agent, very useful and clinically important drugs have been developed. The most important is Lovastatin which was first reported by Merck from *Aspergillus terreus.*

The best known and widely used immunosuppressive compound of fungal origin is cyclosporine, a metabolic product of *Tolypocladivum inflatum*. Orignally discovered as an antifungal agent from this fungus besides *Cylindrocarpon lucidum*. It was subsequently discovered to have excellent immunosuppressive activity and useful for the treatment for organ transplants. The cyclosporins represent a group of biologically active secondary metabolites from *Tolypocladivum* as well as other genera such as *Beauveria, Fusarium, Paecilomyces and Verticillium.* Mycophenolic acid is another example

of a fungal based immunosuppressant. It was initially discovered from *Penicillium brevicompactum* in 1896 and subsequently found in a number of other penicillia. A metabolite with immunosuppressive activity was discovered from *Isaria sinclarii*.

Search for antiviral activity is planned by understanding the biochemical events that predispose the onset of viral infection. For example, during HIV replication, a series of complex events including specific binding with viral RNA and with viral and host protein is involved. The viral protein has a regulatory role in the transport of viral RNA into host cytoplasm and it is envisaged that blocking this interaction could yield antiviral compounds. Scientists at the Bristol Myers Squibb Laboratories identified from *Trichoderma harzianum* compounds designated Harziphilone and Fleephilone which exhibited such inhibitory activities.

Fungal metabolites active against protozoa, nematodes and insects have received widespread attention and interest. Cures have been sought among microbial metabolites for Protozoan infections like amoebiasis due to *Entamoeba histolyitca* and coccidiosis in chick caused by *Eimeria tenella*. Antiamoebin, a peptide isolated from *Emericellopsis synnematicola* and related species has shown high activity against these pathogens and has been demonstrated to be effective against other protozoan parasites like *Trichomonas vaginalis* and *Trypanosoma evansii*. Tabata *et al*, 1995 isolated Fudecalone from a *Penicillium* sp. which was a terpene type metabolite which was inhibitory to *Eimeria tenella*[23].

Nematode trapping fungi and their possible role in the control of plant pathogenic nematodes destructive to crop plants has also received attention. Predaceous fungi like *Arthrobotrys, Dactyella* and *Dactylaria* have been studied mycologically as possible biocontrol agents. From *Arthrobotrys oligospora*, oligosporons have been identified which are nematicidal metabolites.

Insecticidal metabolites have also been widely studied among which the polyoxins and nikkomycins produced by actinomycetes are useful for control of insects by virtue of their ability to inhibit chitin synthesis. It has been observed that fungal scleroctia which help the fungi to tide over unfavourable conditions in the natural environment elaborate compounds which are insect repellants or insecticidal. A group of compounds known as Aflavines have been identified from sclerotia of several *Aspergillus* species as well as from the ascostroma of *Eupenicillium crustaceum*. Cyclodepsipeptides which are toxic to insects and other invertebrates have been identified from several entomogenous fungi and include Beauvericin from *Beauveria bassiana*[24]. *Alternaria alternata* produces a metabolite termed Tentoxin and this is a phytotoxin with herbicidal activity. Search for fungal metabolites having vasodilator activity and prevention of platelet aggregation has led to the discovery of some interesting compounds. A new alkaloid, amauromine has been isolated from the culture broth of *Amauroascus* with vasodialating activity.

Many fungi produce toxic compounds which adversely affect human and animal and these toxic metabolites are termed mycotoxins. Aflatoxin produced by *Aspergillus flavus* is one of the most stable carcinogenic compounds produced in groundnut and a variety of other food materials. Other examples of mycotoxins include, Zearalenone from *Fusarium graminearum* which causes vulvovaginitis and infertility in cattle and pigs. Trichothecenes are another group of toxin produced by different species of *Fusarium*. Slatramine is a toxin from *Rhizoctonia leguminicola* causing excessive salivation in cattle referred to as “Slobber Syndrome”. Islandicin and Luteoskyrin are carcinogenic toxins from *Penicillium islandicum* that cause hepatitis in humans. The epothilones, isolated from *Myxomycetes* (gliding bacteria) have drawn the eyes of many researchers as a potential anti-tumor agent[25]. Ergot consists of dried sclerotia of the fungus *Claviceps purpurea* parasitic on rye. Although, ergot powder was used to hasten childbirth by midwives for thousands of years,

its use in modern medicine was first reported by German physician Lonicer in 1582. The first scientific report of ergot as a cytotoxic agent was made by the American physician Starns in 1808. However, the most important alkaloids, which are therapeutically important, are ergometrine, ergometrinine, ergotamine, ergosine, ergocryptine, ergocrystine, etc. In addition, a number of other alkaloids of minor nature are also present. Ergometrine derivatives are mostly used to stop haemorrhage after childbirth. Ergotamine is used against migraine. A derivative of ergometrine is also used in migraine. Ergotoxine group of alkaloids in equal parts have been used for controlling essential hypertension and other peripheral disorders[8].

BIOACTIVE METABOLITES FROM ENDOPHYTES

An endophyte is a bacterial or fungal microorganism, which reside inside the healthy tissues of the host plant typically causing no damage to them. Endophytic fungi mimic the chemistry of the respective host plant and produce the same bioactive natural products or derivatives as their host plant[26]. Since the discovery of endophytes in Darnel, Germany, in 1904, various investigators have defined endophytes in different ways, which is usually dependent on the perspective from which the endophytes were being isolated and subsequently examined. Bacon and White gave an inclusive and widely accepted definition of endophytes-"microbes that colonize living, internal tissues of plant without causing any immediate, overt negative effects"[27]. While the symptomless nature of endophytic occupation in plant tissue has prompted focus on symbiotic or mutualistic relationships between endophytes and their hosts, the biodiversity of endophytes suggest that they can also be aggressive endophytes or opportunistic pathogens. Fungi and bacteria are the most common microbes existing as endophytes. Between them fungi are the most commonly isolated one. It turns out that the vast majority of plants have not been studied for their endophytes. Thus enormous opportunities exist for recovery of novel fungal forms, taxa and biotypes. It is estimated that there may be as many as 1 million different fungal species, yet only about 100,000 have been described[28]. It seems obvious that endophytes are a rich and reliable source of genetic diversity and novel, undescribed species.

Almost all vascular plant species examined to date were found to harbor endophytic bacteria or fungi[29]. Moreover, the colonization of endophytes in marine algae, mosses and ferns has been recorded. As a matter of fact, endophytes are important components of microbial diversity[30]. Commonly, several to thousands of endophyte species can be isolated from a single plant, and amongst them, at least one species showing specificity. The environmental conditions under which the host is growing, also effect the endophytic population and the endophyte population can be more diversified in tropical areas. Moreover, genotypic diversity has been observed in single endophytic species originating from conifers, birch and grasses. Accordingly, endophytes are ubiquitous in the plant kingdom with the population being dependent on host species and location.

Some phytopathogens in the environment are of endophyte origins[31]. During the long co-evolution of the phytopathogen and its host plant, an endophytic mutant may result from balanced antagonism and/or gene mutation. Dual cultures of the host calli and endophytes demonstrated that both the endophytes and the host calli excrete metabolites toxic to each other[32]. Further investigation led to the development of a hypothesis that the endophyte– host interaction could be a balanced pathogen–host antagonism. Freeman and Rodriguez[33] found that a naturally occurring nonpathogenic endophytic mutant developed from the mutation of a single locus in the genome of the wild-type *Colletotrichum magna*, a pathogen causing anthracnose in cucurbit plants. This mutant is able to grow systemically inside the host plant without pathogenic symptoms, but retaining wild-type levels of *in vitro* sporulation, spore adhesion, appressoria formation, infection and host

specificity. The *Acremonium* (asexual fungi now reclassified in the genus *Neotyphodium* Glenn, Bacon and Hanlin)[34] endophytes, which usually inhabit tall fescue, perennial ryegrass (*Lolium perenne* L.), and many cool-season grasses, are considered mutualistic symbionts of the host grasses. The grass and the endophytic fungus are so intimately associated that they act 'as a whole', much like 'a single organism'. And, indeed, some of these endophytic *Neotyphodium* species can only spread by infecting seeds from the mother plants[35]. Endophytes colonizing inside plant tissues usually get nutrition and protection from the host plant. In return, they confer profoundly enhanced fitness to the host plants by producing certain fungal metabolites. Endophyte infected plants often grow faster than non-infected ones[36]. This effect is at least in part due to the endophytes production of phytohormones such as indole-3-acetic acid (IAA), cytokines, and other plant growth-promoting substances and/or partly owing to the fact that endophytes could enhance the uptake of nutritional elements such as nitrogen and phosphorous[37].

Certain endophytes improve the ecological adaptability of hosts by enhancing their tolerance to environmental stresses and resistance to phytopathogens and/or herbivores including some insects feeding on host plant. Endophyte infected grasses posses an increased tolerance to drought and aluminium toxicity[38]. Furthermore, some endophytes are able to provide the host plant with protection against some nematodes[39], mammals[40]and insect herbivores[41] as well as bacterial and fungal pathogens. Some endophytes are capable of enhancing the hosts allelopathic effects on other species co-growing nearby, usually being competitor for the nutrition and the space. This could be the reason why some plants with special endophytes are usually competitive enough to become dominant species in successional fields[42,43]. We have isolated, purified and identified an endophytic fungus *Fusarium solani* from the Indian *Catharanthus roseus* plant which produces high valued drugs vinblastine and vincristine[44,45,46].

CONCLUSIONS

The objective of this paper was to review the diversity of bioactive metabolites with medicinal value produced by microorganism, from the interdisciplinary perspectives of biochemistry, biotechnology, genetics, fungal biology etc.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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